

Supplemental Methods

RP-HPLC/LTQ-FT MS/MS, MASCOT search and protein identification criteria. In-gel tryptic digestion was performed as previously described (1). Samples were introduced into a hybrid linear quadrupole ion trap Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (LTQ-FT, ThermoFisher Scientific, Waltham, MA) via capillary liquid chromatography (RP-HPLC). Peptides were separated on a 0.32×50 mm MicroTech Scientific reverse-phase column at a flow rate of 5–10 μ l/min with a linear gradient from 5 to 65% acetonitrile in 0.06% aqueous formic acid (v/v) over 55 min using a LC Packings Ultimate Chromatograph (Dionex, Sunnyvale, CA). Survey MS spectra (m/z 300–2000) were obtained using dynamic exclusion and acquired in the FT-ICR cell with resolution $R = 25,000$ at m/z 400 and accumulation to a target value of 5×10^5 charges or a maximum ion accumulation time of 2000 ms. The dynamic exclusion duration was 200 sec and set to expire if the ion intensity fell below a S/N threshold of 2. The three most intensive ions were fragmented in the linear ion trap by collisionally-induced dissociation (target value of 2×10^3 accumulated ions) and an ion selection threshold of 3000 counts. The ESI source was operated with a spray voltage of 2.8 kV, a tube lens offset of 170 V and a capillary temperature of 200 °C. All other source parameters were optimized for maximum sensitivity of a synthetic YGGFL peptide ion at m/z 556.27.

The resulting raw files were processed with MaxQuant (v. 1.0.13.13, www.maxquant.org) (2) and the three output files were submitted to Mascot (v. 2.2, Matrix Science, London, United Kingdom) for peptide and protein identification against a concatenated forward-target and reverse-decoy International Protein Index (IPI) rat database (v 3.52) supplemented with common contaminants. Search parameters specified a MS tolerance of 30 ppm, a fragment tolerance of 0.5 Da, up to 2 missed cleavages, 3 labeled amino acids per peptide and carboxyamidomethylated cysteine as a fixed modification. Variable modifications were set to consider methionine oxidation, protein N-terminal acetylation, Gln to pyro-Glu and Glu to pyro-Glu. It is not necessary to specify the isotopic labels as variable modifications when using the MaxQuant software (3).

For identification, peptides were required to be at least 7 amino acids in length and proteins were deemed identified if they had at least one unique peptide. If two SILAC pairs were quantified for a protein between any two treatment groups, the protein was included in the analysis. The false discovery rates were set to 5% at the peptide level and 1% at the protein level. Posterior error probabilities (PEP, false hit probability given the peptide score and length) (2) ranged from 0 to 0.027 and after mass recalibration, the average absolute mass deviation was 1.88 ppm.

Protein quantification and bioinformatic analyses. MaxQuant provides an accurate quantitative assessment of a given SILAC pair and reports the median light to heavy ratio obtained from all the peptides associated with a protein. Protein quantification is highly accurate if 3 or more peptides are used (2) and proteins quantified by two or fewer peptides were manually verified before inclusion in the dataset. Since the culture-derived isotope tags serve as the internal standard in each treatment, it was canceled out by dividing the control ratio into those obtained from the diabetic or diabetic + insulin

treated animals (4) (Fig. 1). The resulting value represents the effect of treatment relative to the control. To determine the threshold for statistical significance, proteins showing at least a 20 or 25% increase or decrease were grouped and compared to the entire dataset using a Kruskal-Wallis non-parametric ANOVA and Dunn's multiple comparison test. This analysis indicated that a minimum difference of 25% was necessary for a value to be considered statistically different from the data set.

Proteins were annotated with respect to localization and molecular function using the Gene Ontology (GO) feature of MaxQuant or manually. Over-represented biologic and toxicologic processes in the dataset were determined using Ingenuity Pathways Analysis. In some instances, over-represented biological processes in subsets of the data were determined using the Biological Networks GO (BinGO) plugin (5) of Cytoscape (6) against the *Rattus norvegicus* reference set. GO terms with $p < 0.005$ using the HyperGeometric test after correcting for multiple term testing by the Benjamini & Hochberg False Discovery Rate were deemed enriched.

References

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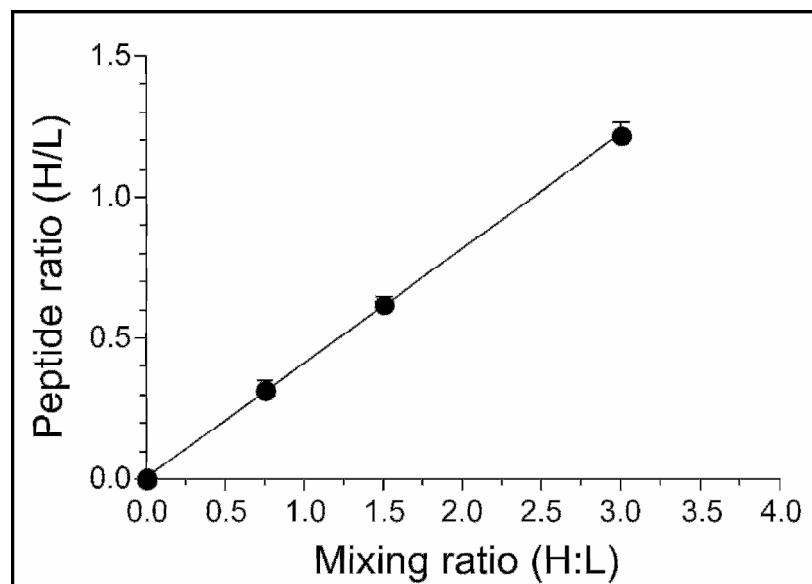
Supplemental Table 1. List of all Proteins Identified and Quantified. (see Online Appendix 2)

Supplemental Table 2. Enzymatic activities of mitochondrial respiratory chain complexes and Kreb's cycle enzyme, citrate synthase are decreased in isolated mitochondria from lumbar DRG of STZ-diabetic rats.

	Enzymatic activity (nmol/min/mg protein)	
Enzymes	Control	Diabetic
Complex I	182.29 ± 14.67	135.73 ± 21.55 *
Complex IV	1488.32 ± 155.44	1262.66 ± 99.19 *
Citrate synthase	239.94 ± 57.91	164.22 ± 18.86*

Enzymatic activity of complex I was assessed as rotenone-sensitive portion of NADH: cytochrome *c* reductase activity. Complex IV activity was measured at 550 nm following the rate of oxidation of reduced cytochrome *c* and the activity of citrate synthase by following the color of thionitrobenzoic acid at 412 nm. Values are means ± SD, n = 4-6. *P<0.05 vs control (Student's *t*-Test).

Supplemental FIG. 1. Validation of response linearity over a 4-fold dynamic range using known ratios of K6R10 to K0R0 mitochondria. K6R10 and K0R0 mitochondrial preparations were mixed in ratios of 0.75:1, 1.5:1 and 3:1 and processed for RP-HPLC/LTQ-FT MS/MS analysis. The K6/K0 and R10/R0 peptide ratios were obtained from the MaxQuant evidence file for each mixture, averaged and plotted against the known mixing ratio. Results are mean ± SEM and represent 78, 188 and 282 peptides from the 0.75:1, 1.5:1 and 3:1 mixtures, respectively.



Supplemental FIG. 2. Representative peptide mass spectra showing the effect of diabetes on expression of ATP synthase alpha. The upper and lower spectra show the doubly charged ion of the unlabeled (m/z 644.35, blue) and K6 labeled (m/z 647.36, black) HALIYDDLK peptide of ATP synthase alpha from a control and diabetic animal, respectively. Since the peptide is doubly charged, the mass difference is 3 atomic mass units and the other colored peaks represent the isotopic envelope of the monoisotopic peak.

